REMARKS/ARGUMENTS

Claims 28-32 are currently pending in the instant application.

Claim Rejections Under 35 U.S.C. §§101 and 112, First Paragraph (Enablement)

Claims 28-32 remain rejected under 35 U.S.C. §101 and 112, first paragraph, allegedly "because the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility", and therefore one skilled in the art clearly would not know how to use the claimed invention. (Page 2 of the instant Final Office Action).

Applicants strongly disagree and, therefore, respectfully traverse the rejection.

Applicants submit that the data presented in Example 143 of the specification, and the cumulative evidence of record, indeed support a "specific, substantial and credible" asserted utility for the presently claimed invention. Applicants rely upon the gene amplification data of the PRO1293 gene for patentable utility of the claimed antibodies to the PRO1293 polypeptide. This data is clearly disclosed in the instant specification in Example 143, which discloses that the gene encoding PRO1293 showed significant amplification in primary lung and colon tumors. As disclosed in previous responses on record, Applicants submit that one of skill in the art would reasonably expect in this instance, based on the amplification data for the PRO1293 gene, that the PRO1293 polypeptide is concomitantly over expressed and that antibodies to PRO1293 have utility in the diagnosis of lung and colon cancer or for individuals at risk for developing lung or colon cancer.

Applicants submit that a ΔCt value of at least 1.0 was observed for PRO1293 in at least three of the tumors listed in Table 8. Table 8 teaches that the nucleic acids encoding PRO1293 showed approximately 1.71 ΔCt units which corresponds to $2^{1.71}$- fold amplification or 3.27-fold amplification in a primary lung tumor (HF-000840), and approximately 1.13-2.33 ΔCt units which corresponds to $2^{1.13}$ -$2^{2.33}$- fold amplification or 2.19 fold to 5.03-fold amplification in colon tumors (HF-000539 and HF-000795). (See Table 8 and page 507, lines 5-12 of the specification) Accordingly, the present specification clearly discloses overwhelming evidence that the gene encoding the PR01293 polypeptide is significantly amplified in lung and colon tumors.

As further support for their utility claim, Applicants have submitted a Declaration by Dr. Audrey Goddard (made of record in the Response submitted August 19, 2004), which explains that a gene identified as being amplified at least 2-fold by the disclosed gene amplification assay in a tumor sample relative to a normal sample is useful as a marker for the diagnosis of cancer, and for monitoring cancer development and/or for measuring the efficacy of cancer therapy. Therefore, such a gene is useful as a marker for the diagnosis of lung cancer, and for monitoring cancer development and/or for measuring the efficacy of cancer therapy. According to the Goddard Declaration, the 2.19 fold to 5.03-fold amplification of the PRO1293 gene in lung and colon tumors would be considered significant and credible by one skilled in the art, based upon the facts disclosed therein. The Examiner has not provided any evidence to show that the disclosed DNA amplification is not significant.

The Examiner asserts that basis of the rejections is solely that gene amplification levels are not predictive of mRNA or polypeptide levels. (Page 3 of the instant Final Office Action).

Applicants have submitted ample evidence to show that, in general, if a gene is amplified in cancer, it is more likely than not that the encoded protein will be expressed at an elevated level. For instance, the articles by Orntoft *et al.*, Hyman *et al.*, and Pollack *et al.* (submitted with Response of August 19, 2004) collectively teach that in general, gene amplification increases mRNA expression.

Further, Applicants have submitted Declarations of Dr. Paul Polakis (made of record Response of August 19, 2004 and Preliminary Amendment of June 2, 2006), which teach that, in general, there is a correlation between mRNA levels and polypeptide levels.

Applicants further submit that even if there were no correlation between gene amplification and increased mRNA/protein expression, (which Applicants expressly do not concede to), a polypeptide encoded by a gene that is amplified in cancer would still have a specific, substantial, and credible utility. Applicants submit that, as evidenced by the Ashkenazi Declaration and the teachings of Hanna et al. (made of record in the Response submitted August 19, 2004), simultaneous testing of gene amplification and gene product over-expression enables more accurate tumor classification, even if the gene-product, the protein, is not over-expressed. This leads to better determination of a suitable therapy for the tumor, as demonstrated by a real-world example of the breast cancer marker HER-2/neu.

Taken together, although there are some examples in the scientific art that do not fit within the central dogma of molecular biology that there is generally a positive correlation between DNA, mRNA, and polypeptide levels, in general, in the majority of amplified genes, as exemplified by the teachings of Orntoft *et al.,* Hyman *et al.,* Pollack *et al.,* and the Polakis Declaration, the art in general overwhelmingly show that gene amplification influences gene expression at the mRNA and protein levels. Therefore, one of skill in the art would reasonably expect in this instance, based on the amplification data for the PRO1293 gene, that the PRO1293 polypeptide is concomitantly overexpressed and has utility in the diagnosis of lung and colon cancers.

Accordingly, Applicants submit that when the proper legal standard is applied, one should reach the conclusion that the present application discloses at least one patentable utility for the claimed antibodies to PRO1293 polypeptides.

The Examiner asserts that "[o]nly two lung cancer samples and one colon sample out of the couple of dozen samples tested positive. Therefore, if a sample were taken from an individual with lung cancer or colon cancer for diagnosis, ***it is more likely*** *than not that this assay would yield a false negative result." (Pages 6-7 of the instant Final Office Action, emphasis in original).*

Applicants emphasize that they have shown significant DNA amplification in three of the lung tumor samples in Table 8, Example 143 of the instant specification. The fact that not all lung and colon tumors tested positive in this study does not make the gene amplification data less significant. As any skilled artisan in the field of oncology would easily appreciate, not all tumor markers are generally associated with every tumor, or even, with most tumors. For example, the article by Hanna and Mornin (of record), discloses that the known breast cancer marker HER-2/neu is "amplified and/or overexpressed in 10%-30% of invasive breast cancers and in 40%-60% of intraductal breast carcinoma" (page 1, col. 1). In fact, some tumor markers are useful for identifying rare malignancies. That is, the association of the tumor marker with a particular type of tumor lesion may be rare, or, the occurrence of that particular kind of tumor lesion itself may be rare. In either event, even these rare tumor markers which do not give a positive hit for most common tumors, have great value in tumor diagnosis, and consequently, in tumor prognosis. The skilled artisan would certainly know that such tumor markers are useful for better

classification of tumors. Therefore, whether the PRO1293 gene is amplified in two lung tumors or in all lung tumors is not relevant to its identification as a tumor marker, or its patentable utility. Rather, the fact that the amplification data for PRO1293 is considered significant is what lends support to its usefulness as a tumor marker.

The Examiner asserts that "Hanna evidences that the level of protein expression must be tested empirically to determine whether or not the protein can be used as a diagnostic marker for a cancer." The Examiner further asserts that Hanna teaches that HER-2/neu testing will utilize FISH and IHC to better establish a correlation. (Pages 12-13 of the Final Office Action).

Applicants respectfully point out that the Examiner appears to have misread Hanna *et al.* Hanna *et al.* clearly state that gene amplification (as measured by FISH) and polypeptide expression (as measured by immunohistochemistry, IHC) are well correlated ("in general, FISH and IHC results correlate well" (Hanna *et al.* p. 1, col. 2)). It is only a subset of tumors which show discordant results. Thus Hanna *et al.* support Applicants' position that it is more likely than not that gene amplification correlates with increased polypeptide expression. Further, the authors make clear that the screening strategy is "based upon the above considerations," that is, the selection of patients who should receive treatment with Herceptin, as discussed in the immediately preceding paragraph. Thus the purpose of measuring both protein and gene levels is not merely further experimentation, but further characterization of the tumors into medically relevant categories.

Applicants have clearly shown that the gene encoding the PRO1293 polypeptide is amplified in at least three primary lung and colon tumors. Therefore, the PRO1293 gene, similar to the HER-2/neu gene disclosed in Hanna *et al.*, is a tumor associated gene. Furthermore, as discussed above, in the majority of amplified genes, the teachings in the art overwhelmingly show that gene amplification influences gene expression at the mRNA and protein levels. Therefore, one of skill in the art would reasonably expect in this instance, based on the amplification data for the PRO1293 gene, that the PRO1293 polypeptide is concomitantly overexpressed.

However, even if gene amplification does not result in overexpression of the gene product (*i.e.*, the protein) an analysis of the expression of the protein is useful in determining the course of treatment, as supported by the Ashkenazi Declaration and the Hanna article. The

Examiner appears to view the testing described in the Ashkenazi Declaration and the Hanna article as experiments involving further characterization of the PRO1293 polypeptide itself. In fact, such testing is for the purpose of characterizing not the PRO1293 polypeptide, but the tumors in which the gene encoding PRO1293 is amplified. The claimed antibodies to the PRO1293 polypeptide are therefore useful in tumor categorization, the results of which become an important tool in the hands of a physician enabling the selection of a treatment modality that holds the most promise for the successful treatment of a patient.

The Examiner addresses the pooled blood controls used in the gene amplification assay and asserts that the controls were not matched, non-tumor lung and colon samples.. (Page 16 of the instant Final Office Action)

Applicants respectfully submit that the Examiner's position is incorrect because the instant application relies on **genomic DNA** amplification for utility and not cDNA expression. Different types of cells from the same organism should have the same set of genomic DNA. Thus, it does not matter what kind of cells you use for the control as long as the control cells have the entire genome. Accordingly, a "tissue-matched" control is not necessary in the gene amplification assay.

Applicants further point out that Pennica *et al.* teaches the exact same "pooled normal blood controls" as that used in the instant gene amplification assay (for instance, see page 14718, column 1 and Figure 5 of Pennica *et al.*). Further, the references Bieche *et al.* and Pitti *et al.*, submitted as Exhibits F and G with the Goddard Declaration, also used "pooled normal blood controls" as control. For instance, in Pitti *et al.* the authors used the same quantitative TaqMan PCR assay and pooled normal blood controls described in the instant specification, to study gene amplification in lung and colon cancer of DcR3, a decoy receptor for Fas ligand. Pitti *et al.* analyzed DNA copy number "in genomic DNA from 35 primary lung and colon tumors, relative to pooled genomic DNA from peripheral blood leukocytes (PBL) of 10 healthy donors." (Page 701, col. 1). The authors also analyzed mRNA expression of DcR3 in primary tumor tissue sections and found tumor-specific expression, confirming the finding of frequent amplification in tumors, and confirming that the pooled blood sample was a valid negative control for the gene amplification experiments. In Bieche *et al.*, the authors used the quantitative TaqMan PCR assay to study gene amplification of myc, ccnd1 and erbB2 in breast tumors. As their negative control,

Bieche *et al.* used normal leukocyte DNA derived from a small subset of the breast cancer patients (page 663). The authors note that "[t]he results of this study are consistent with those reported in the literature" (page 664, col. 2). Thus, contrary to the Examiner's allegations, Pennica *et al,* Pitti *et al.* and Bieche *et al.* in fact, confirm the validity of use of the "pooled blood control" as a negative controls, and indicate that this control was widely utilized in the art at the time of filing of the instant application.

The Examiner has also asserted that the data presented in the specification were not corrected for aneuploidy and cites references by Hittelman et al., Fleischhacker et al. and Sen et al. in support of the assertion that "[a] slight amplification of a gene does not necessarily correlate with overexpression in a cancer tissue, but can merely be an indication that the cancer tissue is aneuploid." (Pages 7-9 of the instant Final Office Action).

Applicants submit that it is known in the art that detection of gene amplification can be used for cancer diagnosis regardless of whether the increase in gene copy number results from intrachromosomal changes or from chromosomal aneuploidy. As explained by Dr. Ashkenazi in his Declaration (submitted with Applicants' Response filed August 19, 2004),

> An increase in gene copy number can result not only from intrachromosomal changes but also from chromosomal aneuploidy. It is important to understand that detection of gene amplification can be used for cancer diagnosis even if the determination includes measurement of chromosomal aneuploidy. Indeed, as long as a significant difference relative to normal tissue is detected, it is irrelevant if the signal originates from an increase in the number of gene copies per chromosome and/or an abnormal number of chromosomes.

Hence, Applicants submit that gene amplification of a gene, whether by aneuploidy or any other mechanism, is useful as a diagnostic marker.

Regarding Sen, Fleischhacker and Hittelman, Applicants agree that while aneuploidy can be a feature of damaged tissue as well, besides cancerous or pre-cancerous tissue, and may not invariably lead to cancer, Sen *et al.* in fact support the Applicants' position that PRO1293 is still useful in diagnosing pre-cancerous lesions or cancer itself. For instance, the art in lung cancer at the time of filing of the instant application clearly described that "epithelial tumors develop through a multistep process driven by genetic instability" in damaged lung lesions which may eventually lead to lung cancer. Many articles published around the effective filing date of this

application studied such damaged or premalignant lesions and suggested that identification of such pre-cancerous lesions were very important in preventive diagnosis and treatment of lung cancer. Based on the well-known art, Applicants submit that there is utility in identifying genetic biomarkers in epithelial tissues at cancer risk.

The Examiner asserts that "[i]n order for PRO1293 polypeptides to be overexpressed in tumors, amplified genomic DNA would have to correlate with increased mRNA levels and increased polypeptide levels. No data regarding PRO1293 mRNA or PRO1293 polypeptide levels in lung or colon tumors have been brought forth on the record." (Pages 7-8 of the instant Final Office Action).

The Examiner's reference to the lack of necessary correlation or accurate prediction in some of the rejections clearly shows that the Examiner applies an improper legal standard when making this rejection. The evidentiary standard to be used throughout *ex parte* examination in setting forth a rejection is a preponderance of the totality of the evidence under consideration. Thus, to overcome the presumption of truth that an assertion of utility by the Applicant enjoys, the Examiner must establish that it is more likely than not that one of ordinary skill in the art would doubt the truth of the statement of utility. Only after the Examiner has made a proper *prima facie* showing of lack of utility, does the burden of rebuttal shift to the Applicant. As discussed below, the references cited by the Examiner do not suffice to make a *prima facie* case that more likely than not no generalized correlation exists between gene (DNA) amplification and increased polypeptide levels.

In contrast, Applicants have submitted ample evidence to show that, in general, if a gene is amplified in cancer, it is more likely than not that the encoded protein will be expressed at an elevated level. First, the articles by Orntoft *et al.*, Hyman *et al.*, and Pollack *et al.*, (made of record in Applicants' Response filed August 19, 2004) collectively teach that in general, gene amplification increases mRNA expression. Second, as the Examiner has acknowledged, the art teaches that, in general, there is a correlation between mRNA levels and polypeptide levels.

Accordingly, one of skill in the art would reasonably expect in this instance, based on the amplification data for the PRO1293 gene, that the PRO1293 polypeptide is concomitantly overexpressed. Thus, the claimed antibodies to the PRO1293 polypeptide have utility in the diagnosis of cancer.

The Examiner asserts that "[s]ignificant further research would have been required of the skilled artisan to reasonably confirm that PRO1293 is overexpressed in any cancer to the extent that it could be used as a cancer diagnostic agent; thus the asserted utility is not substantial." (Page 10 of the instant Final Office Action).

As discussed in previous responses of record, M.P.E.P. §2107.01 cautions Office personnel not to interpret the phrase "immediate benefit to the public" or similar formulations used in certain court decisions to mean that products or services based on the claimed invention must be "currently available" to the public in order to satisfy the utility requirement. "Rather, any reasonable use that an Applicant has identified for the invention that can be viewed as providing a public benefit should be accepted as sufficient, at least with regard to defining a 'substantial' utility."[1] Indeed, the Guidelines for Examination of Applications for Compliance With the Utility Requirement,[2] gives the following instruction to patent examiners: "If the Applicant has asserted that the claimed invention is useful for any particular practical purpose . . . and the assertion would be considered credible by a person of ordinary skill in the art, do not impose a rejection based on lack of utility."

Applicants' position is based on the overwhelming evidence from gene amplification data disclosed in the specification which clearly indicate that the gene encoding PRO1293 is significantly amplified in certain lung and colon tumors. Based on the working hypothesis among those skilled in the art that if a gene is amplified in cancer, the encoded protein is likely to be expressed at an elevated level, one skilled in the art would simply accept that since the PRO1293 gene is amplified, the PRO1293 polypeptide would be more likely than not over-expressed. Thus, data relating to PRO1293 polypeptide expression may be used for the same diagnostic and prognostic purposes as data relating to PRO1293 gene expression. Therefore, based on the disclosure in the specification, no further research would be necessary to determine how to use the claimed PRO1293 polypeptides or antibodies thereto, because the current invention is fully enabled by the disclosure of the present application.

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[1] M.P.E.P. §2107.01.

[2] M.P.E.P. §2107 II(B)(1).

Accordingly, Applicants submit that based on the general knowledge in the art at the time the invention was made and the teachings in the specification, the specification provides clear guidance as to how to interpret and use the data relating to PRO1293 polypeptide expression and that the claimed PRO1293 antibodies have utility in the diagnosis of cancer.

A *prima facie* case of lack of utility has not been established

Applicants respectfully submit that the Examiner has not made a proper *prima facie* showing of lack of utility, because the Examiner has not shown that Applicants' asserted utility is more likely than not incorrect.

The Examiner asserts that "[t]he art discloses that a correlation between genomic DNA levels and mRNA levels cannot be presumed, nor can any correlation between genomic DNA levels and polypeptide levels", citing Pennica, Konopka, Sen, Hittelman, Godbout and Li (pages 8-10 of the instant Final Office Action).

As a preliminary matter, Applicants respectfully submit that it is not a legal requirement to establish that gene amplification "necessarily" results in increased expression at the mRNA and polypeptide levels. As discussed in the previous responses of record, the evidentiary standard to be used throughout *ex parte* examination of a patent application is a preponderance of the totality of the evidence under consideration. Accordingly, Applicants submit that in order to overcome the presumption of truth that an assertion of utility by the applicant enjoys, the Examiner must establish that **it is more likely than not** that one of ordinary skill in the art would doubt the truth of the statement of utility. Therefore, it is not legally required that there be a "necessary" correlation between the data presented and the claimed subject matter. The law requires only that one skilled in the art should accept that such a correlation is **more likely than not to exist**. Applicants respectfully submit that when the proper evidentiary standard is applied, a correlation must be acknowledged.

Applicants have previously cited Orntoft *et al.*, Hyman *et al.*, and Pollack *et al.* as collectively teaching that in general, gene amplification increases mRNA expression. Further, Applicants' arguments directed to the references cited by the Examiner presented in the previously filed Responses are hereby incorporated by reference in their entirety.

Pennica *et al.*

The Examiner has cited the abstract of Pennica *et al.* for its disclosure that "WISP-2 DNA was amplified in colon tumors, but mRNA expression was significantly reduced in the majority of tumors compared with the expression in normal colonic mucosa from the same patient." (Page 8 of the instant Office Action). From this, the Examiner has concluded that increased copy number does not *necessarily* result in increased polypeptide expression. The standard, however, is not absolute certainty. The fact that in the case of a specific class of closely related molecules there seemed to be no correlation with gene amplification and the level of mRNA/protein expression, does not establish that it is more likely than not, in general, that such correlation does not exist.

Nowhere in the Pennica paper does the author suggest that it is more likely than not that altered mRNA levels does not correlate with altered protein levels. On the contrary, there is a statement in Pennica that says "[a]n analysis of *WISP*-1 gene amplification and expression in human colon tumors *showed a correlation between DNA amplification and over-expression...*" (Pennica *et al.*, page 14722, left column, first full paragraph, emphasis added), which implies that the mRNA/protein correlation does exist, even if not always, but "always" is not required by the utility standard.

The Examiner has not shown whether the lack or correlation observed for the family of WISP polypeptides is typical, or is merely a discrepancy, an exception to the rule of correlation. Indeed, the working hypothesis among those skilled in the art is that, if a gene is amplified in cancer, the encoded protein is likely to be expressed at an elevated level. In fact, as noted even in Pennica *et al.*, "[a]n analysis of *WISP*-1 gene amplification and expression in human colon tumors *showed a correlation between DNA amplification and over-expression* . . . ." (Pennica *et al.*, page14722, left column, first full paragraph, emphasis added). Accordingly, Applicants respectfully submit that Pennica *et al.* teaches nothing conclusive regarding the absence of correlation between amplification of a gene and over-expression of the encoded WISP polypeptide. More importantly, the teaching of Pennica *et al.* is specific to *WISP* genes. Pennica *et al.* has no teaching whatsoever about the correlation of gene amplification and protein expression in general.

Konopka *et al.*

The Examiner has also cited the abstract of Konopka et al. to establish the assertion that protein expression is not related to gene amplification but to variation in the level of mRNA produced from a single genomic template. (Page 8 of the instant Final Office Action).

Regarding Konopka *et al.*, Applicants submit that the Examiner has completely misinterpreted the statement that "[p]rotein expression is not related to amplification of the *abl* gene but to variation in the level of *bcr-abl* mRNA produced from a single Ph^1 template." (See Konopka *et al* ., Abstract). Konoka teaches that "[t]he demonstration that the Ph^1 chromosomal template can vary in its level of expression of P210 $^{c\text{-}abl}$ suggests that secondary mechanisms, beyond the translocation itself, contribute to the regulation of the *bcr-abl* gene in different cell types or subclones that derive from the affected stem cell." (page 4049, 2nd column, 2nd paragraph) In an effort to characterize this differential expression, Konopka examined the production of the *abl* RNA via RNA blot hybridization analysis (Fig. 3), which "showed that the normal 6- and 7-kb *c-abl* mRNAs were present at a similar level in Ph^1-positive and -negative cell lines derived from different patients." (page 4050, 2nd column, last paragraph) However, Konopka found that "the 8-kb mRNA that encodes P210 $^{c\text{-}abl}$ was detected at a 10-fold higher level in SK-CML7Bt-33 (Fig. 3A, +) than in SK-CML16Bt-1 (B, +), which correlated with the relative level of P210 $^{c\text{-}abl}$ detected in each cell line. Analysis of additional cell lines demonstrated that the level of 8-kb RNA directly correlated with the level of P210 $^{c\text{-}abl}$ (Table 1)" (page 4050, 2nd. column, last paragraph; underlining added). As a further control, Konopka looked at DNA levels and found that "[t]he variation in level of 8-kb RNA detected in these cell lines was not due to loss or gain of Ph^1, because cytogenetic analysis confirmed the presence of Ph^1 in these cell lines." (page 4050, 2nd column, last paragraph) Konopka further established that "[t]here was no difference in the copy number of abl-related sequences as judged by Southern blot analysis (Fig. 4)." (page 4051, 1st column) From this study, Kopoka concludes "[t]hese combined data suggest that differential bcr-abl mRNA expression from a single gene template is responsible for the variable levels of P210 $^{c\text{-}abl}$ detected." (page 4051, 1st column) Therefore, the teachings of Konopka *et al.* are not pertinent to the examiner's argument because they are not directed towards the correlation of gene amplification and its gene product other than to demonstrate that there are mechanisms other than gene amplification that contribute to protein

overexpression in cancer. However, Kopoka does support Applicants' position regarding a correlation between mRNA and protein levels.

Godbout *et al.*

Regarding Godbout, the Examiner has asserted that Godbout et al. teaches that "a number of studies suggest that co-amplified genes are only overexpressed if they provide a selective advantage to the cells in which they are amplified." The Examiner further asserts that Godbout teaches "[i]t is generally accepted that co-amplified genes are not over-expressed unless they provide a selective growth advantage to the cell." (Page 9 of the instant Final Office Action).

Applicants have previously made of record three more recent references, published in 2002, by Ornstoft *et al.*, Hyman *et al.*, and Pollack *et al*, (made of record in Applicants' Response filed on August 19, 2004), which collectively teach that in general, gene amplification increases mRNA expression. Applicants submit that these more recent references must be acknowledged as more accurately reflecting the state of the art regarding the correlation between gene amplification and transcript expression than the references cited by Godbout *et al.*

Applicants further maintain that Godbout *et al.* report that "there is a good correlation with DDX1 gene copy number, DDX1 transcript levels, and DDX1 protein levels in all cell lines studied." Thus, in these cancer cell lines, DDX1 mRNA and protein levels are correlated.

Moreover, selective advantage to cell survival is not the only mechanism by which genes impact cancer. Mechanistic data is not a requirement for the utility requirement. Hence, this rejection is improper. Applicants respectfully submit that, as discussed above, Ornstoft *et al.*, Hyman *et al.*, and Pollack *et al.*, (of record), collectively teach that gene amplification increases mRNA expression for large numbers of genes, which have not been identified as being oncogenes or as conferring any selective growth advantage on tumor cells. Thus, the art of record clearly shows that there is no requirement that a polypeptide must be a known oncogene or a protein otherwise known to be associated with tumor growth, in order for amplification of the gene encoding the protein to correlate with increased protein expression. In fact, as demonstrated by Ornstoft *et al.*, Hyman *et al.*, and Pollack *et al.*, examination of gene

amplification is a useful way to identify novel proteins not previously known to be associated with cancer.

Li *et al.*

The Examiner also cites Li et al. as teaching that "68.8% of the genes showing over-representation in the genome did not show elevated transcript levels." (Page 10 of the instant Final Office Action).

Applicants respectfully point out that Li *et al.* acknowledge that their results differed from those obtained by Hyman *et al.* and Pollack *et al.* (of record), who found a substantially higher level of correlation between gene amplification and increased gene expression. The authors note that "[t]his discordance may reflect methodologic differences between studies or biological differences between breast cancer and lung adenocarcinoma" (page 2629, col. 1). In fact, as explained in the Supplemental Information accompanying the Li article, genes were considered to be amplified if they had a copy number ratio of at least 1.40. As discussed in Applicants' previous responses, and in the Goddard Declaration of record, an appropriate threshold for considering gene amplification to be significant is a copy number of at least 2.0. As discussed above, the PRO1293 gene showed 2.19 fold to 5.03-fold amplification in adenocarcinomas or squamous cell carcinomas of the lung and colon, thus meeting this standard. It is not surprising that, by using a substantially lower threshold for considering a gene to be amplified, Li *et al.* would have identified a number of genes that were not in fact significantly amplified, and therefore did not show any corresponding increase in mRNA expression. The results of Li *et al.* therefore do not disprove that a gene with a substantially higher level of gene amplification, such as PRO1293, would be expected to show a corresponding increase in transcript expression.

In summary, the Patent Office has failed to meet its initial burden of proof that Applicants' claims of utility are not substantial or credible. The arguments presented by the Examiner in combination with the cited articles do not provide sufficient reasons to doubt the statements by Applicants that PRO1293 has utility. As discussed above, the law does not require that DNA amplification is "always" associated with overexpression of the gene product. Therefore, Applicants submit that the Examiner's reasoning is based on a misrepresentation of

the scientific data presented in the above cited reference and application of an improper, heightened legal standard. In fact, contrary to what the Examiner contends, the art indicates that, if a gene is amplified in cancer, it is more likely than not that the encoded protein will be expressed at an elevated level.

It is "more likely than not" for amplified genes to have increased mRNA and protein levels

Applicants have submitted ample evidence to show that, in general, if a gene is amplified in cancer, it is more likely than not that the encoded protein will be expressed at an elevated level. First, the articles by Ornthoft *et al.*, Hyman *et al.*, and Pollack *et al.*, (of record) collectively teach that in general, gene amplification increases mRNA expression.

Second, as the Examiner has acknowledged, the art teaches that, in general, there is a correlation between mRNA levels and polypeptide levels.

Thus, taken together, all of the submitted evidence supports Applicants' position that gene amplification is more likely than not predictive of increased mRNA and polypeptide levels.

The Examiner asserts that "the rejection is no longer based on the issue of whether or not mRNA levels are predictive of protein levels. Therefore, these findings of Orntoft et al. are no longer relevant to the rejection." (Page 16 of the instant Final Office Action).

Applicants submit that Orntoft was submitted to show that there was a gene dosage effect and teaches that "in general (18 of 23 cases) chromosomal areas with more than 2-fold gain of DNA showed a corresponding increase in mRNA transcripts" (see column 1, abstract). Based on this reference along with Hyman and Pollack, Applicants have submitted that it is generally well-understood in the art that DNA copy number influences gene expression. For example, Orntoft *et al.* studied transcript levels of 5600 genes in malignant bladder cancers which were linked to a gain/loss of chromosomal material using an array-based method.

The Examiner asserts that "Orntoft et al. could only compare the levels of about 40 well-resolved and focused abundant proteins." (Page 16 of the instant Final Office Action).

While technical considerations did prevent Orntoft *et al.* from evaluating a larger number of proteins, the ones they did look at showed a clear correlation between mRNA and protein expression levels. As Orntoft *et al.* states, "In general there was a highly significant correlation ($p<0.005$) between mRNA and protein alterations.... 26 well focused proteins whose genes had

a known chromosomal location were detected in TCCs 733 and 335, and of these 19 correlated ($p<0.005$) with the mRNA changes detected using the arrays." (See page 42, column 2 to page 34, column 2). Accordingly, Orntoft *et al.* clearly support Applicants' position that proteins expressed by genes that are amplified in tumors are useful as cancer markers.

As discussed in previous responses, the levels of amplification for PRO1293 were **not** "low" but significant, and ranged from 2.0-fold to 3.05-fold, in three different lung tumors. Applicants note that the levels of gene amplification observed by Orntoft *et al.* were relatively low, averaging only 0.3-0.4-fold (page 40, col. 1). In particular, the level of gene amplification associated with expression changes was only around two-fold (see Figure 2), even less than the 2.0-fold to 3.05-fold amplification observed for PRO1293. Even with these relatively low levels of gene amplification, Orntoft *et al.* found that "**[i]n most cases**, chromosomal gains detected by CGH were accompanied by an increased level of transcripts in both TCCs 733 (77%) and 827 (80%)" (page 40, col. 2; emphasis added). The level of correlation between DNA copy number and increased mRNA levels observed by Orntoft *et al.*, from **77-80%**, clearly meets the standard of more likely than not. Orntoft *et al.* also found a "highly significant" correlation between mRNA and protein levels, with the two data sets studied having correlations of 39/40 (**98%**) and 19/26 (**73%**) (pages 42-43).

With respect to the evidence provided by Orntoft et al., the Examiner asserts that "Orntoft et al. only concentrated on regions of chromosomes with strong gains of chromosomal material containing clusters of genes (p.40). This analysis was not done for PRO1293 in the instant specification. That is, it is not clear whether or not PRO1293 is in a gene cluster in a region of a chromosome that is highly amplified." (Pages 16-17 of the instant Final Office Action).

Applicants fail to see how this is relevant to the analysis. Orntoft *et al.* did not limit their findings to only those regions of amplified gene clusters. Further, as discussed in Applicants' previous Responses Hyman *et al.* and Pollack *et al.* did gene-by-gene analysis across all chromosomes.

Applicants note that Orntoft *et al.* also studied the relation between altered mRNA and protein levels using 2D-PAGE analysis, and that this analysis was done on a gene by gene basis, with the authors selecting 40 well resolved abundant known proteins for which to assess the

correlation between mRNA and protein levels for each gene. The authors found that "[i]n general **there was a highly significant correlation ($p<0.005$) between mRNA and protein alterations**. Only one gene [of the 40 examined] showed disagreement between transcript alteration and protein alteration" (page 42, col. 2; emphasis added). Clearly, a correlation in 39 of 40 genes examined supports Applicants' assertion that changes in mRNA level generally lead to corresponding changes in protein level.

The Examiner further asserts that the Hyman reference teaches "[l]ess than half (44%) of highly amplified genes showed mRNA overexpression (abstract)." (Page 17 of the instant Final Office Action).

Applicants submit the Examiner's assertion is not consistent with the interpretation Hyman *et al.* themselves place on their data, stating that, "The results illustrate **a considerable influence of copy number on gene expression patterns**." (page 6242. col. 1; emphasis added). In the more detailed discussion of their results, Hyman *et al.* teach that "[u]p to 44% of the highly amplified transcripts (CGH ratio, >2.5) were overexpressed (***i.e.*, belonged to the global upper 7% of expression ratios**) compared with only 6% for genes with normal copy number." (See page 6242, col. 1; emphasis added). These details make it clear that Hyman *et al.* set a highly restrictive standard for considering a gene to be overexpressed; yet almost half of all highly amplified transcripts met even this highly restrictive standard. Therefore, the analysis performed by Hyman *et al.* clearly shows that "it is more likely than not" that a gene which is amplified in tumor cells will have increased gene expression.

The Examiner has alleged that "Pollack et al also used CGH technology, concentrating on large chromosome regions showing high amplification (p. 12965). Pollack et al. did not investigate polypeptide levels. Therefore, Pollack et al. also do not support the asserted utility of the claimed invention." (Page 17 of the instant Final Office Action).

Applicants maintain that Pollack *et al.* profiled DNA copy number alteration across 6,691 mapped human genes in 44 predominantly advanced primary breast tumors and 10 breast cancer cell lines. Pollack *et al.* further state, "Parallel microarray measurements of mRNA levels reveal the remarkable degree to which variation in gene copy number contributes to variation in gene expression in tumor cells." (See Abstract). "Genome-wide, of 117 high-level DNA amplifications (fluorescence ratios >4, and representing 91 different genes), 62% (representing

54 different genes; ...) are found associated with at least moderately elevated mRNA levels (mean-centered fluorescence ratios >2), and 42% (representing 36 different genes) are found associated with comparably highly elevated mRNA levels (mean-centered fluorescence ratios >4)." (See page 12966, column 1). Therefore, the analysis performed by Pollack *et al.* was also on a gene-by gene basis, and clearly shows that "it is more likely than not" that a gene which is amplified in tumor cells will have increased gene expression.

As stated above, the Orntoft *et al.,* Hyman *et al.,* and Pollack *et al.* articles were submitted to support the correlation between gene amplification and mRNA levels, which according to the Examiner is the sole basis of the maintained rejections. With regard to the correlation between mRNA expression and protein levels, Applicants previously submitted a Declaration by Dr. Polakis, principal investigator of the Tumor Antigen Project of Genentech, Inc., the assignee of the present application, to show that mRNA expression correlates well with protein levels, in general.

Applicants also resubmit herein over 100 references (Applicants count an abstract and the full length article as one reference) in addition to the declarations and references already of record which support Applicants' asserted utility, either directly or indirectly. These references support the argument that, in general, a change in mRNA expression level for a particular gene leads to a corresponding change in the level of expression of the encoded protein. Applicants refer to the arguments and information presented in their Preliminary Amendment of March 9, 2007 in response to the outstanding utility rejection, wherein those arguments are incorporated by reference herein. As Applicants have acknowledged, the correlation between changes in mRNA level and protein level is not exact, and there are exceptions (*see, e.g.,* abstracts submitted in the IDS herein as Exhibit 21). However, Applicants remind the PTO that the asserted utility does not have to be established to a statistical certainty, or beyond a reasonable doubt. *See M.P.E.P.* at § 2107.02, part VII (2004). Therefore, the fact that there are exceptions to the correlation between changes in mRNA and changes in protein does not provide a proper basis for rejecting Applicants' asserted utility. Applicants submit that considering the evidence as a whole, with the overwhelming majority of the evidence supporting Applicants' asserted utility, a person of skill in the art would conclude that Applicants' asserted utility is "more likely than not true." *Id.*

The Examiner asserts that of the 118 additional references cited by Applicants in their Preliminary Amendment filed June 2, 2006, "none of the cited references address the major issue in this rejection, which is whether or not the 1293 gene amplification in lung and colon tumor leads to overexpression of the PRO1293 polypeptide in said tumors. (Page 19 of the Final Office Action).

Applicants have acknowledged that the new references cited in the Preliminary Amendment filed June 2, 2006 and included in the IDS submitted herein, focus on the correlation between mRNA expression and protein expression levels, and for the most part do not examine gene amplification. However, those few references that actually looked at gene amplification **did** find a correlation between gene amplification and increased mRNA and protein expression levels. Applicants further respectfully submit that, as discussed in the Preliminary Amendment filed June 2, 2007, Godbout *et al.* (J. Biol. Chem. 1998; 273(33)21161-8) (abstract attached as Exhibit 14) studied the DEAD box gene, DDX1, in retinoblastoma and neuroblastoma tumor cell lines. The authors report that "there is a good correlation with DDX1 gene copy number, DDX1 transcript levels, and DDX1 protein levels in all cell lines studied." *Id.* Thus, in these cancer cell lines, DDX1 mRNA and protein levels are correlated.

In addition, Bea *et al.* (Cancer Res. 2001; 61(6):2409-12) (abstract attached in Exhibit 12) investigated gene amplification, mRNA expression, and protein expression of the putative oncogene BMI-1 in human lymphoma samples. The authors found BMI-1 gene amplification in four mantle cell lymphomas (MCLs). Bea *et al.* report that "[t]he **four tumors with gene amplification showed significantly higher mRNA levels** than other MCLs and NHLs with the BMI-1 gene in germline configuration" (Abstract; emphasis added). Applicants note that the fact that five additional MCLs also showed very high mRNA levels without gene amplification does not disprove Applicants' position, because one of skill in the art would understand that there can be more than one cause of mRNA overexpression. The issue is not whether mRNA overexpression is always, or even typically caused by gene amplification, but rather, whether gene amplification typically leads to overexpression. Bea *et al.* further note that the four MCLS with gene amplification of *BMI-1* "showed significantly higher levels of mRNA **and protein expression** compared with other lymphomas with *BMI-1* in germline configuration" (page 2411,

col. 1; emphasis added). Thus Bea *et al.* supports Applicants' assertion that gene amplification is correlated with both increased mRNA and protein expression.

With regard to the correlation between gene amplification, mRNA expression and protein levels, the Examiner has asserted that the Polakis Declaration and the decision by the Board of Patent Appeals and Interferences are insufficient to overcome the rejection of claims 28-36 and 38-40 since they are limited to a discussion of data regarding the correlation of mRNA levels and polypeptide levels and not gene amplification levels. (Page 18 of the instant Final Office Action).

Applicants submit that Dr. Polakis' Declaration and the decision by the Board of Patent Appeals and Interferences were presented to support the position that there is a correlation between mRNA levels and polypeptide levels, the correlation between gene amplification and mRNA levels having already been established by the data shown in the Orntoft *et al.*, Hyman *et al.*, and Pollack *et al.* articles. Applicants emphasize that the opinions expressed in the Polakis Declaration, including the quoted statement, are all based on factual findings. Thus, Dr. Polakis explains that in the course of their research using microarray analysis, he and his co-workers identified approximately 200 gene transcripts that are present in human tumor cells at significantly higher levels than in corresponding normal human cells. Subsequently, antibodies binding to about 30 of these tumor antigens were prepared, and mRNA and protein levels were compared. In approximately 80% of the cases, the researchers found that increases in the level of a particular mRNA correlated with changes in the level of protein expressed from that mRNA when human tumor cells are compared with their corresponding normal cells. Dr. Polakis' statement that "an increased level of mRNA in a tumor cell relative to a normal cell typically correlates to a similar increase in abundance of the encoded protein in the tumor cell relative to the normal cell" is based on factual, experimental findings, clearly set forth in the Declaration. Accordingly, the Declaration is not merely conclusive, and the fact-based conclusions of Dr. Polakis would be considered reasonable and accurate by one skilled in the art.

The Examiner has further asserted that the declaration "does establish the correlation between a change, if any, in PRO1293 transcripts and PRO1293 polypeptide expression in tumors because there are examples of genes for which such a correlation does not exist." (Page 18 of the instant Final Office Action).

The standard, however, is not absolute certainty. The fact that there may be examples which demonstrate no correlation with gene amplification and the level of mRNA/protein expression, does not establish that it is more likely than not, in general, that such correlation does not exist.

Taken together, although there are some examples in the scientific art that do not fit within the central dogma of molecular biology that there is a correlation between polypeptide and mRNA levels, these instances are exceptions rather than the rule. In the majority of amplified genes, the teachings in the art, as exemplified by Orntoft *et al.*, Hyman *et al.*, Pollack *et al.*, and the Polakis Declaration, overwhelmingly show that gene amplification influences gene expression at the mRNA and protein levels. Therefore, one of skill in the art would reasonably expect in this instance, based on the amplification data for the PRO1293 gene, that the PRO1293 polypeptide is concomitantly overexpressed. Thus, Applicants submit that the claimed antibodies to the PRO1293 polypeptide have utility in the diagnosis of cancer.

Applicants therefore respectfully request withdrawal of the rejections of Claims 28-32 under 35 U.S.C. §101 and 35 U.S.C. §112, first paragraph.

CONCLUSION

In conclusion, the present application is believed to be in *prima facie* condition for allowance, and an early action to that effect is respectfully solicited. Should there be any further issues outstanding, the Examiner is invited to contact the undersigned attorney at the telephone number shown below.

The Commissioner is hereby authorized to charge any fees, including any fees for extension of time, or credit overpayment to Deposit Account No. **07-1700**, referencing Attorney's Docket No. **123851-181898 (39780-2830 P1C4)**.

Please direct any calls in connection with this application to the undersigned at the number provided below.

Respectfully submitted,

Date: August 20, 2008

By: _____
Christopher De Vry (Reg. No. 61,425)

Goodwin Procter LLP
Customer No. 77845
135 Commonwealth Drive
Menlo Park, CA 94025
T: 650.752.3100
F: 650.853.1038

LIBC/3362611.1